ZOOLOGICAL SCIENCE 10: 543–546 (1993)

[RAPID COMMUNICATION]

Neural Control of Muscle Differentiation in the Leg of the Fleshfly *Sarcophaga bullata*

Pakkirisamy Sivasubramanian

Department of Biology, University of New Brunswick, Fredericton, Canada E3B 6E1

ABSTRACT—The tubular leg muscles of an adult fly differentiate from embryonic adepithelial cells present within the leg imaginal discs. Transection of the disc stalk from the ventral ganglion in a prepupa results in the formation of an externally normal looking leg that lacks locomotor muscles. The absence of motor axons in the nerve of in the experimental leg is suggestive of the importance of motor innervation in the development of leg muscles.

INTRODUCTION

Several studies using both *in vitro* and *in vivo* procedures have demonstrated the importance of innervation in the differentiation of vertebrate muscles [3, 6, 9, 14, 15, 19]. Among invertebrates insects have received special attention in this regard. The influences of nerves on insect muscle differentiation was first demonstrated by Kopec [8]. When he removed thoracic ganglia from gypsy moth caterpillars the resulting adults were devoid of thoracic muscles. Similar results were also obtained for silkmoths [11, 21]. By a more refined procedure Sivasubramanian and Nässel [18] recently demonstrated the role of mesothoracic larval nerve in the differentiation of thoracic flight muscles in the fly, *Sarcophaga bullata*. Although these studies did indicate the importance of innervation for the differentiation of flight muscles, additional information can be obtained from examining leg muscle differentiation in holometabolous insects such as flies which arise *de novo* from bags of embryonic cells called imaginal discs [4, 13, 20]. This is in contrast to the thoracic flight muscles that differentiate using the histolysing larval muscles as templates [5]. Further, the imaginal discs are attached to the larval ventral ganglion by means of stalks which also contain nerves that apparently act as guides for the growing adult axons between the legs and the ventral ganglion [1]. In larger species of flies these disc stalks along with their guiding pioneer axons can be surgically severed. The present study examines the legs developed from stalk transected imaginal discs of the fleshfly, *Sarcophaga bullata*. The results strongly suggest the importance of motor innervation for leg muscle differentiation.

MATERIALS AND METHODS

The fleshfly *Sarcophaga bullata* was reared in the laboratory under constant conditions of temperature (25°C) and photoperiod (16L:8D). Adults were fed with ample supply of sugar and water. Larvae were raised in fresh beef liver. Post-feeding, mature third instar larvae were collected from culture containers and used for experiments. All operations were performed on freshly formed prepupae (1–2 hr post pupariation). Mesothoracic legs lacking motor innervation were produced according to the method of Sivasubramanian and Nässel [17]. Since the stalk of the leg disc contains axons that presumably act as pioneers to guide the adult nerves disconnecting the stalk deprives the growing motor neurons from finding their targets. A "V" shaped incision was made on the anterior

Accepted April 12, 1993
Received February 4, 1993

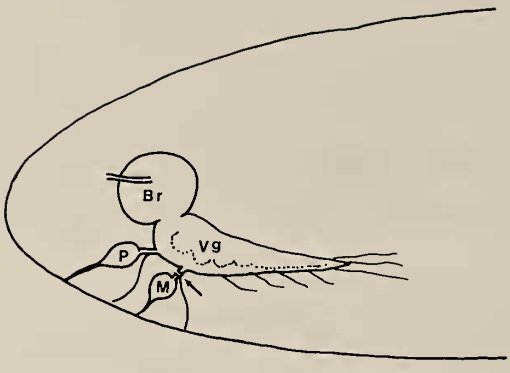

Fig. 1. Schematic diagram showing the lateral view of the larval brain (Br) and ventral ganglion (Vg). M and P are, respectively, mesothoracic and prothoracic leg imaginal discs. The arrow depicts the location of stalk transection.

ventral side (on segments 5 and 6, i.e., 2nd and 3rd segments after anterior spiracles) of freshly formed prepupa. The triangular flap was lifted up and the exposed stalk of left mesothoracic leg disc was transected. The site of transection is shown in Figure 1. The flap was left back on the slit and the dried hemolymph closed twe wound. The operated specimens kept in humid chambers at 25°C. metamorphosed into normal looking flies in 12 days.

Twenty experimental (left mesothoracic) legs were examined histologically and compared with normal, right mesothoracic legs. The femurs of the legs were embedded in epon and 2 μm thick plastic sections were stained with toluidine blue.

RESULTS AND DISCUSSION

Morphologically, the legs developed from stalk transected imaginal discs appear quite normal like the control legs (Fig. 2A and C) with full complement of segments, bristles etc. However, these experimental legs exhibit very little movement. The flies simply drag these legs while walking. Examination of histological sections reveal the reason for the absence of motor function in these legs. A cross section of the femur of a normal (right) mesothoracic leg is shown in Figure 2B. It is filled with tibial levator and depressor muscles. Compared to this the experimental leg is devoid of both sets of these muscles (Fig. 2D). There is a nerve bundle in the operated leg (Fig. 2D, arrow). Our previous study of horseradish peroxidase fillings of such stalk transected legs [17] as well as the absence of any movement in these legs suggest that this nerve is probably composed mostly sensory axons. However, these histological and behavioral observations cannot rule out the presence of few, albeit, nonfunctional motor axons in the nerve bundle.

Within the imaginal discs there are irregular shaped adepithelial cells which are the progenitors of adult leg muscles [13]. During metamorphosis, these cells organize themselves into columns, become multinucleate and eventually differentiate into the tubular muscles of the leg [16]. Are these adepithelial cells dependent on motor innervation for their differentiation?

The results of earlier studies were ambiguous about the role of innervation on leg muscle differentiation. When *Drosophila* mesothoracic leg discs were cultured *in vivo* muscle specific enzymes were expressed in the transplanted leg discs [20]. However, when the discs were cultured *in vitro* muscles failed to differentiate in the otherwise morphologically normal looking legs [9]. The present study used a different approach to examine the same problem. Here an *in situ* leg disc was deprived of motor innervation by transecting the disc stalk prior to metamorphosis [17]. When the stalk was selectively severed leaving the disc's attachment with the epidermis an externally normal looking leg developed. It is quite conceivable that the absence of muscle in the stalk transected legs is due to the lack of motor axons in the nerves of these legs.

Myogenesis is dependent on motor innervation in chick embryos [3]. Destruction of motor neurons by bungarotoxin inhibits myotube formation in rat skeletal muscles [6]. Presumably, the motor neuron terminals have some trophic or inductive influence on myogenic cells [15]. In grasshopper embryos motor neurons contact muscle pioneers very early in development [2] and perhaps this contact influences them from the very beginning. Precisely what stage of leg muscle differentiation in flies is controlled by motor innervation is not clear at the moment. However, preliminary screening of histological preparations

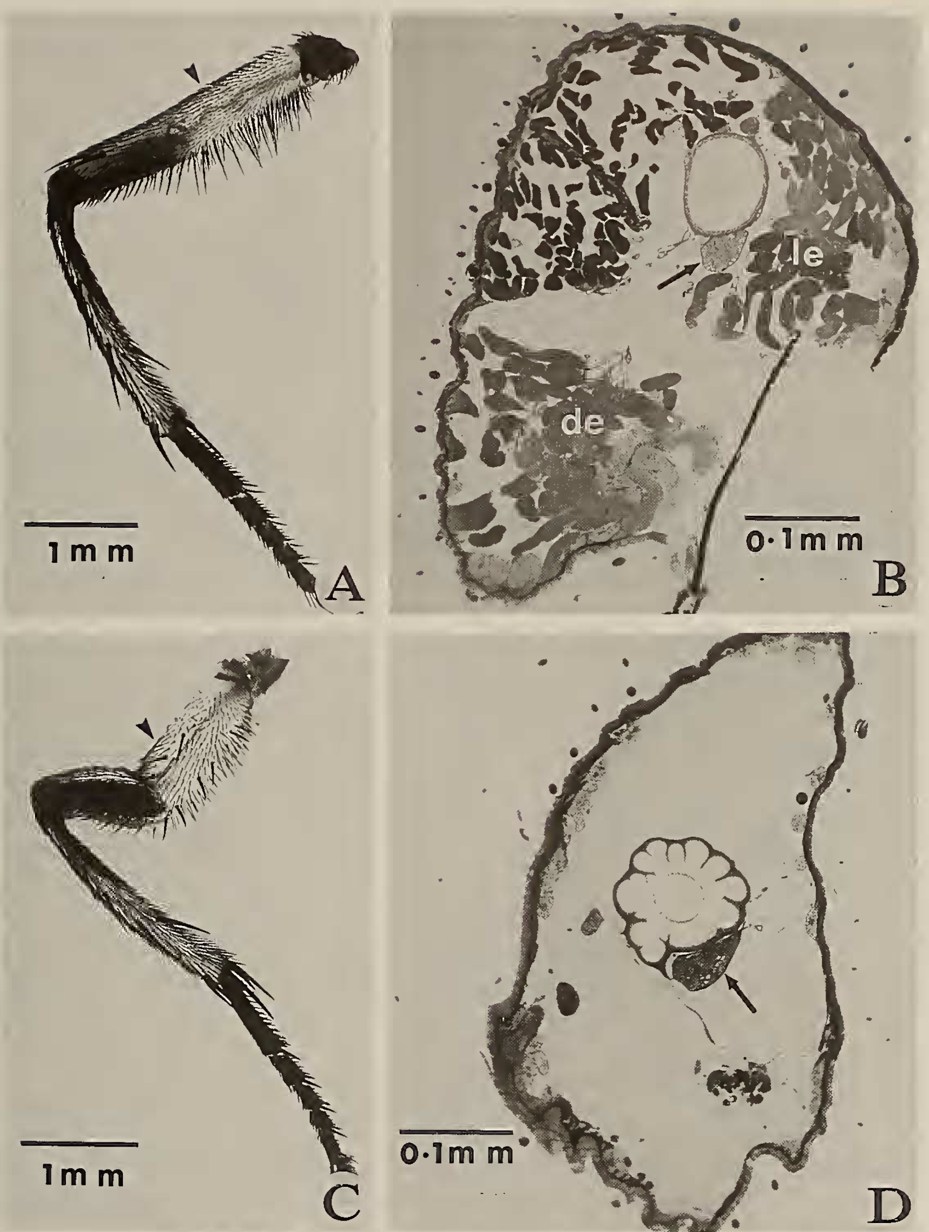

FIG. 2. Control (A) and stalk transected (C) legs indicating the approximate site of cross section (arrow heads). B. Cross section of a normal leg with levator (le) and depressor (de) muscles. D. Cross section of a stalk transected leg. Note the leg is completely devoid of muscles. Both legs have a leg nerve (arrow).

of developing stalk transected legs indicates that the proliferation of adepithelial cells (myoblast precursors) is unaffected implying the post mitotic events of myogenesis to be highly dependent on motor innervation.

ACKNOWLEDGMENTS

This research was supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

1 Auerbach C (1936) Trans Roy Soc Edin 58: 787–815
2 Ball EE, Ho RK, Goodman CS (1985) J Neurosci 5: 1808–1819
3 Bonner PH, Adams TR (1982) Develop Biol 90: 174–184
4 Crossley AC (1978) In "The Genetics and Biology of *Drosophila* Vol 2b" Ed by M Ashburner, TRF Wright, Academic Press, pp 499–560
5 Fernandes J, Bate M, Vijayraghavan K (1991) Development 113: 67–77
6 Harris AJ (1981) Phil Trans Roy Soc Lond Ser B. 293: 257–277
7 Jan YN, Ghysen A, Christoph I, Barbel S, Jan LY (1985) J Neurosci 5: 2453–2464
8 Kopec C (1923) J Exp Zool 37: 15–25
9 Mandaron P (1970) Develop Biol 22: 298–320
10 McLennan IS (1983) Develop Biol 98: 287–294
11 Nässel DR (1983) In "Functional Neuroanatomy" Ed by NJ Strausefeld, Springer Verlag, pp 44–91
12 Nüesch H (1985) In "Comprehensive Insect Physiology, Biochemistry and Pharmacology Vol 2" Ed by G Kerkut, LI Gibert, Pergamon Press, pp 425–452
13 Poodry CA, Schneiderman HA (1970) Roux's Arch Dev Biol 166: 1–44
14 Popiela H (1978) Exp Neurol 62: 404–416
15 Popiela H, Ellis S (1981) Develop Biol 83: 266–277
16 Reed CT, Murphy C, Fristrom D (1975) Roux's Arch Dev Biol 178: 285–302
17 Sivasubramanian P, Nässel DR (1989) Develop Growth Diff 31: 341–349
18 Sivasubramanian P, Nässel DR (1990) Zool Sci 7: 223–228
19 Sohal GS, Holt RK (1980) Cell Tiss Res 210: 383–393
20 Ursprung HM, Conscience-Egli M, Fox DJ, Wallimann T (1972) Proc Nat Acad Sci USA 69: 2812–2813
21 Williams CM, Schneiderman HA (1952) Anat Rec 113: 560–561